

In re of Appln. No. 09/830,954
Amendment under 37 C.F.R. §1.312
March 7, 2005

Amendments to the Specification

Please replace the paragraph [0100] with the following amended paragraph [0100]:

[0100] According to another aspect of the present invention there is provided an agent for treating a plaque forming disease. The agent according to this aspect of the present invention comprising a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

Please replace the paragraph [0103] with the following amended paragraph [0103]:

[0103] Use of beta amyloid peptide antigens in conjunction with adjuvants to effect immunization has previously been difficult due to the resulting combination of high toxicity and low titers which result. Using prior art methods as a starting point, immunization of a mouse with a 16 amino acids peptide of beta-amyloid conjugated to KLH (SEQ ID NO:9) was carried out. This immunization produced a low but measurable antibody titer against beta-amyloid.

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Please replace the paragraph [0103] with the following amended paragraph [0103]:

[0126] In the early stages of AD, the BBB may limit the entry of antibody circulating in the periphery to the CNS. In contrast A β anti-aggregating antibodies displayed on a phage surface have the potential not only to be delivered directly to the CNS by intranasal administration but also to prevent olfactory permanent damage by A β in the patients. As previously shown, intranasal administration (Mathison et al, 1998; Chou et al, 1997; Draghia et al, 1995) enables the direct entry of viruses and macromolecules into the CSF or CNS.

Please replace the paragraph [0210] with the following amended paragraph [0210]:

[210] ScFv-508F fusion to filamentous minor coat gpIII were used in order to investigate the ability of β AP anti-aggregating scFv to be ~~carries~~ carried by a filamentous phage display system directly into the CNS.

Please replace the paragraph [0212] with the following amended paragraph [0212]:

[212] Immediately following decapitation, brains were removed and cut into two halves along the mid-sagittal sinus. Randomly, one half-brain was fixed by immersion in 4% paraformaldehyde solution in 0.1 M phosphate buffer for two

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hours in 4°C and then immersed for cold protection in 4.5% sucrose in 0.1 M PBS over night. The sections were then moved to 30% sucrose for 2 hours in 4°C. Sections of coronal blocks containing the olfactory and hippocampus were put in OCT and cut with ~~thick-nesses~~thicknesses of 6 μ m with a cryostat at -20°C-20°C, and then taken up on glass slides. Slides were kept at -70°C. These slides were used for phage detection using an immunofluorescence technique.

Please replace the paragraph [0213] with the following amended paragraph [0213]:

[213] The other mid-sagittal half-brain was used for preparing paraffin tissue section for histology. The section were fixed in 4% paraformaldehyde for 2 hours, then transferred to 10% formalin saline for 2 days in room temperature, followed by embedding in paraffin, and cut with ~~thick-nesses~~thicknesses of 4 μ m on a microtome and then taken up on glass slides. The slides were kept at room temperature until used.

Please replace the paragraph [0216] with the following amended paragraph [0216]:

[216] Immunizations were performed with a genetically engineered fd phage carrying the peptide YYEFRH (SEQ ID NO:7) fused to its minor coat gpIII. Doses of 10¹⁰ phages per injection were used to immunize at 14-day intervals, through

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intraperitoneal injections. Mice were injected with the phages with or without Freund's complete adjuvant (Difco) for the first injection and Freund's incomplete adjuvant (Difco) for the second injection. Following 7 days of each injections, the mice were bled and their serum were tested by ELISA for antibody IgG reactivity for both phage coat proteins and for β A.

Please replace the paragraph [0223] with the following amended paragraph [0223]:

[223] The inhibition of antibody binding to β AP(1-16) by various small peptides was performed using 250 ng/well biotinylated β -amyloid peptides (1-16) bound covalently to ELISA plates as previously described. The plates were washed with PBS/0.05% Tween 20 and blocked with a mixture of 3% bovine serum albumin and hemoglobin, ratio 1:1 (in PBS) for 2 hours at 37°C. The peptides were pre incubated with 1:3000 dilution of serum after third immunization with f88-EFRH for 30 minutes at 37°C before their addition to β AP-coated wells and were left overnight at 4°C therein. After washing, bound antibody was detected by incubation with HRP-conjugated rabbit anti-mouse immunoglobulin, as described above. The results were used to derive the IC₅₀, which is the half molar concentration of peptide that fully inhibits antibody binding. Peptides were synthesis-synthesized by Applied Biosystems

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Synergy Model 430A in the Unit for Chemical Services of the
Weizmann Institute of Science by solid-phase using Fmoc
chemistry.

Please replace the paragraph [0243] with the
following amended paragraph [0243] :

[243] Female Balb/c mice were treated with phage vector f88-EFRH via intranasal administration. The ~~propose~~ purpose of this experiment was to check the ability of filamentous phage to reach the hippocampus region via olfactory tract. Since the phage is not carrying any specific molecule for targeting neuron cells, it should be vanished without causing any harm after several day following the administration. In order to investigate the appearance of phage in the olfactory bulb and the hippocampus region double labeling of antibodies was used as follows: rabbit polyclonal antibody anti filamentous phage and mouse monoclonal antibody against EFRH (SEQ ID NO:1) epitope fused to glycoprotein VIII of the phage surface. One day following a single intranasal administration of 10^{11} phages animals showed such phages in their olfactory bulb and hippocampus (Figures 14a-d). Seven days after the administration phages were detected in the olfactory bulb of only one mouse of the three tested, whereas no phages were revealed in the hippocampus. No evidence of phages was detectable 28 days following administration. As shown in

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Figures 15a-d, no evidence of change in the neuron population of the brain of treated mice was evident.

Please replace the heading preceding paragraph [0244] with the following amended heading:

EXAMPLE 8: Filamentous Phage Axe Is Suitable Vehicle for Carrying Active Antibody Fragment to the CNS

Please replace the paragraph [0246] with the following amended paragraph [0246]:

[246] The anti-aggregating epitope within β AP (EFRH, SEQ ID NO:1) map to positions 3-6 of the amino acid sequence of β AP. In order to generate specific immune response against β AP, mice were immunized with genetically engineered fd phage carrying the peptide YYEPRH (SEQ ID NO:7) fused to its minor coat gpIII according to the immunization schedule shown in Figure 17. Doses of 10^{10} phage particles per injection were used to immunize, at 14-day intervals, through intraperitoneal injection. Following 7 days of each injection, mice were bled and their sera tested by ELISA for IgG antibody reactivity against wild type phage (not bearing the peptide YYEPRH (SEQ ID NO:7) on its surface) and against β AP (Figures 18a-b).

This route of administration gave a very high response against β AP (1:750) following the third injection. Furthermore, it was found that injection through phage carrying epitope is long lasting (Figure 19), it is non-toxic and may be given without

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adjuvant. The phage vector is found to be an immunogenic tool to raise a high affinity immune response within 14 days from the first injection. The immune response against the peptide YYEFRH (SEQ ID NO:7) is low, compared to the immune response against the entire phage and could be explained by the low copy number of the fusion gpIII on the phage envelope. Therefore, for further analysis phages displaying the epitope through glycoprotein VIII were employed.

Please replace the paragraph [0247] with the following amended paragraph [0247]:

[0247] To identify a disaggregating EFRH (SEQ ID NO:1) peptide epitope a phage-epitope library was screened with biotinylated antibody. After three cycles of panning and phage amplification, 90 individually isolated bacterial colonies were grown in microtiter plates and their phages were assayed for antibody binding. ELISA analysis revealed that of the phage-clones that were selected followed by three biopanning cycles, most (above 80%) bound specifically to anti-aggregating mAb, respectively. DNA from 6 positive clones was sequenced (Table 2). The sequence EFRH (SEQ ID NO:1) appeared in 4 clones, one additional clone had the sequence ~~EFRH~~ (SEQ ID NO:1) EPRH (residues 2-5 of SEQ ID NO:23), with only one residue replacement of proline with phenylalanine. In one additional clone, the inserted peptide bears the sequence of

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the three residues FRH (acids 2-4 of SEQ ID NO:1), lacking the glutamate residue.

Please replace the paragraph [0253] with the following amended paragraph [0253]:

[0200] To examine the effect of serum of f88-EFRH immunized mice on disruption of the β A fibril (the toxic form of β AP) the ThT reagent that binds specifically to fibrillar structures (Levine H. III, 1993) was used. β AP samples were incubated for a week at 37°C and then were exposed to different dilutions of mouse serum antibody. Fibril formation was quantitated by the ThT fluorometry binding assay. Figure 25 shows that mouse serum, at dilution of 1:5 and 1:20, disrupted the fibril structure of β A with extensive deterioration of fibril morphology, as indicated by a substantial 75% (1:5 dilution) and 50% (1:20 dilution) decrease in ThT fluorescence. The unrelated serum used as control (serum from non-immunized mouse), did not significantly inhibit fibril formation as is compared to the immunized serum. This result strongly emphasizes the ability of the EFRH (SEQ ID NO:1) epitope displayed by a filamentous phage vector to evoke an immune response resulting in anti-aggregation antibody.

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